tionsmaxima dem Canthaxanthin ähnliches Pigment, das er wegen seines Vorkommens in Chlorella «Chlorellaxanthin» nannte. Wir konnten diesen Farbstoff aus *Chlorella pyrenoidosa* (Nr. 211–8b der Sammlung PRINGSHEIM, Göttingen) und aus zwei weiteren nicht näher identifizierten, von Baumstämmen in Marburg (Lahn) isolierten Chlorella-Stämmen nach dem üblichen Ausschüttelverfahren säulenchromatographisch gewinnen[1]. Aus einer Methanol/Benzol-Mischung kristallisiert das Pigment in dunkelroten Prismen, die bei 216–218°C (unkorr.) schmelzen. Die physikalischen Daten stimmen mit denen[6–8] von authentischem Canthaxanthin überein. Auch Mischchromatogramme ($CaCO_3$, Al_2O_3, Petroläther, Benzol) und Mischschmelzpunkt mit synthetischem Canthaxanthin beweisen die Identität beider Pigmente.

Damit ist Canthaxanthin neben Astaxanthin das zweite chemisch eindeutig bestimmte Sekundär-Carotinoid in grünen Algen. Dieses von HAXO[6] aus *Cantharellus cinnabarinus* und von SAPERSTEIN und STARR[7] aus einer farbigen Mutante von *Corynebacterium michiganense* isolierte Ketocarotinoid, dessen Struktur von PETRACEK und ZECHMEISTER[8] aufgeklärt wurde, konnte später von VÖLKER[9] in Vogelfedern und kürzlich von THOMMEN und WACKERNAGEL[10] in Daphnien nachgewiesen werden[11].

Summary. The ketonic carotenoid canthaxanthin has been isolated from three nitrogen-starved strains of *Chlorella*.

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[11] Der F. HOFFMANN-LA ROCHE AG, Basel, danke ich für authentisches Canthaxanthin. Mit freundlicher Genehmigung von Herrn Prof. Dr. H. DRAWERT wurden diese Untersuchungen im Botanischen Institut der Universität Marburg durchgeführt. Der Deutschen Forschungsgemeinschaft bin ich für ein Stipendium und für Sachbeihilfen zu Dank verpflichtet.

On the Presence of Sepiapterin Reductase Different from Folate and Dihydrofolate Reductase in Chicken Liver

Concerning the metabolism of sepiapterin (2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine)[1], it was reported that the pteridine was reduced in the presence of reduced nicotinamide dinucleotide phosphate (NADPH) by a folate reductase from chicken liver and Drosophila[2,3]. On the other hand, we showed that in a silkworm two distinct enzymes were responsible for attacking folate and sepiapterin, respectively[4].

In the present communication, we wish to present evidence indicating the presence of sepiapterin reductase different from folate and dihydrofolate reductase in chicken liver.

Experiments. Acetone powder of chicken liver was prepared as described by ZAKRZEWSKI[5]. All subsequent enzyme preparations were performed at 0–3°C, and centrifugation was carried out at 14,000 *g* for 20 min.

Acetone powder (5 g) was stirred for 1 h with 20 vol of cold water. After centrifugation, the supernatant (crude extract) was fractionated with ammonium sulphate. The fractions precipitating between 0.18 and 0.34 saturation (fraction 1) and 0.49 and 0.60 saturation (fraction 2), were collected, redissolved in 5 ml of water and dialysed overnight against distilled water. After removing insoluble materials by centrifugation, the supernatants were used for assay of enzyme. Dihydrofolate reductase was prepared by the method of SILVER et al.[6]. This fraction corresponds to 0.60–0.70 saturation of ammonium sulphate (fraction 3).

Sepiapterin reductase was assayed in the following reaction mixture: McIlvaine buffer (pH 6.0, 100 μmoles), sepiapterin (0.1 μmole), NADPH (0.1 μmole) and enzyme (0.2 ml), brought to a total volume of 0.5 ml. After incubation at 37°C for 30 min, the reaction was stopped by the addition of 0.5 ml of 16% trichloroacetic acid. After centrifugation, the supernatant was neutralized with 0.5 ml of 0.8 *M* tris-(hydroxymethyl)-amino-methane, and then optical density at 420 mμ of the mixture was measured to know the decrease in a concentration of sepiapterin.

Assay of folate reductase was performed in the mixture of citrate buffer (pH 5.0, 40 μmoles), NADPH (0.08 μmole), folate (0.05 μmole) and enzyme (0.3 ml), brought to a total volume of 2.5 ml. After incubation at 37°C for 30 min, the reaction was stopped by the addition of 0.5 ml of 30% trichloroacetic acid, and the amount of the resultant tetrahydrofolate (FAH_4) was determined by the method of BRATTON and MARSHALL[7].

Dihydrofolate reductase was assayed by measuring the decrease in optical density at 340 mμ of the reaction mixture of potassium phosphate buffer (pH 7.5, 50 μmoles), dihydrofolate (0.1 μmole), NADPH (0.1 μmole) and enzyme (0.2 ml), brought to a total volume of 1.2 ml.

Protein was determined by the method of LOWRY et al.[8]. NADPH was prepared enzymatically from NADP[5]. Dihydrofolate was prepared according to FUTTERMAN's method[9]. Purified sepiapterin was obtained from mutant sepia of Drosophila (unpublished work).

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Results and discussion. Table I shows the typical results of the experiments on the enzyme activities of each fraction from chicken liver. The activity of sepiapterin reductase is mostly concentrated in fraction 1 and only a little activity is detectable in fraction 2. Fraction 3, which is freed from folate reductase as reported by SILVER et al.[6], is also devoid of the activity of sepiapterin reductase. These results apparently indicate that sepiapterin reductase is distinguishable from both folate and dihydrofolate reductase. The effect of aminopterin on sepiapterin reductase further supports the above conclusion. As already known, folate and dihydrofolate reductase are completely inhibited by concentration of aminopterin as low as $1 \times 10^{-5} M$, while the inhibitory effect of the drug on sepiapterin reductase is only slight even at the concentration of $1 \times 10^{-4} M$ (Table II).

Table I. Comparison of sepiapterin-, folate- and dihydrofolate-reductase activities in enzyme fraction from chicken liver

Enzyme fraction	Sepiapterin reductase	Folate reductase	Dihydrofolate reductase
	$-\Delta E_{420}$[a]	$\mu g FAH_4$[a]	$-\Delta E_{340}$[a]
Crude extract	0.148	4.15	0.421
Fraction 1	0.320	0.47	0.050
Fraction 2	0.016	13.30	1.190
Fraction 3	0.000	0.00	0.300

[a] per mg protein per h.

Table II. Effect of aminopterin on reductase activities

Final concentration of aminopterin	Sepiapterin reductase	Folate reductase	Dihydrofolate reductase
$1 \times 10^{-5} M$	10	100	100
$1 \times 10^{-4} M$	11	100	100

Numbers in columns show % inhibition.

Other than insect tissues and chicken liver, the activity of sepiapterin reductase is widely distributed in the liver of mammals, such as rat, hog, rabbit, horse and oxen.

On the biochemical role of sepiapterin, it is interesting that the pteridine has high cofactor activity in KAUFMAN's system of phenylalanine-hydroxylation[10]. According to him, tyrosine formation in the presence of sepiapterin[10] or dihydrobiopterin[11] is dependent on dihydrofolate reductase. The present findings conflict with the idea that sepiapterin is reduced directly to tetrahydro level by dihydrofolate reductase to participate in the hydroxylation reaction of phenylalanine. The biochemical role of sepiapterin reductase is currently being investigated and will be reported elsewhere.

Résumé. Nous avons démontré dans le foie de poussin l'existence d'un ferment qui réduit spécifiquement la sépiaptérine. Il est capable de séparer le ferment de réductase d'acide folique et d'acide dihydrofolique après précipitation fractionnaire de sulfate d'ammonium. Le ferment, réductase de sépiaptérine, se trouve largement dans les foies des mammifères, tels que rat, cochon, lapin, cheval et bœuf.

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Monosynaptic Inhibition of the Intracerebellar Nuclei Induced from the Cerebellar Cortex

In the previous report[1], it has been shown that in the neurones of Deiters' nucleus of the cat the inhibitory postsynaptic potentials (IPSPs) are induced *monosynaptically* by stimulation of the cerebellar cortex at the anterior vermian part. It was then suggested that this inhibition may be carried by the long corticofugal fibres which originate from the cerebellar cortex and project directly onto Deiters' neurones. If this were so, the nature of the cerebellar Purkinje cells would be specified as inhibitory. It would follow that a similar inhibition occurs at any target neurones of the corticofugal projection either within or without the cerebellum[2]. In the work to be reported, this postulate was substantiated by the intracellular recording from the intracerebellar nuclei during stimulation of the cerebellar cortex.

Adult cats were used under anaesthesia by pentobarbitone sodium. The procedure of dissection and the experimental arrangement were the same as those employed for the intracellular recording from Deiters' neurones[1,3]. Microelectrodes were inserted into the nuclei dentatus, interpositus and fastigii by way of the pharyngeal approach[3]. As seen in Figure A, they were reached at the depth of 11 to 13 mm along tracks with lateral angle of −5 to 35 degrees (− indicates medial). In order to stimulate antidromically the nuclei interpositus and dentatus, concentric electrodes with the outside diameter of 0.5 mm and interpolar distance of 1 to 1.5 mm were inserted stereotaxically into the red nucleus (RN) and also into the nucleus ventralis lateralis of the thalamus (VL), as shown in Figure B. Concentric electrodes of the same type were inserted into the anterior lobe of the cerebellum stereotaxically[1] or into the cerebellar hemisphere under direct vision. In the latter case three electrodes were implanted at intervals of 3 to 5 mm along the crus II, and their internal poles at 1 to 1.5 mm under the cortical surface were used as cathode against their respective outside

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